Roles of nitrifiers in the removal of micropollutants during wastewater treatment processes

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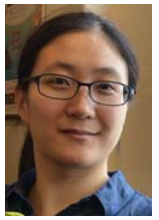
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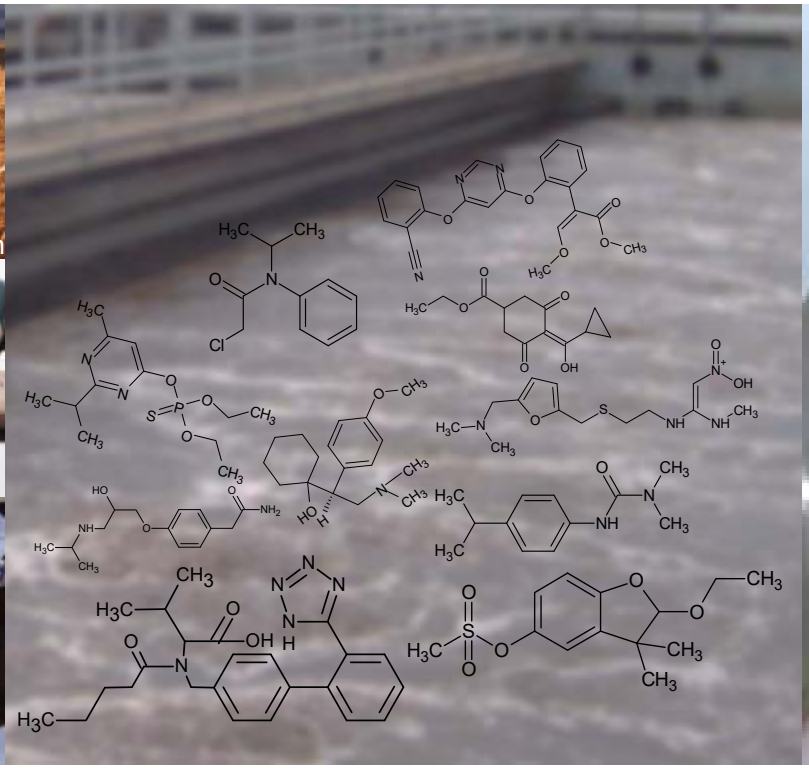
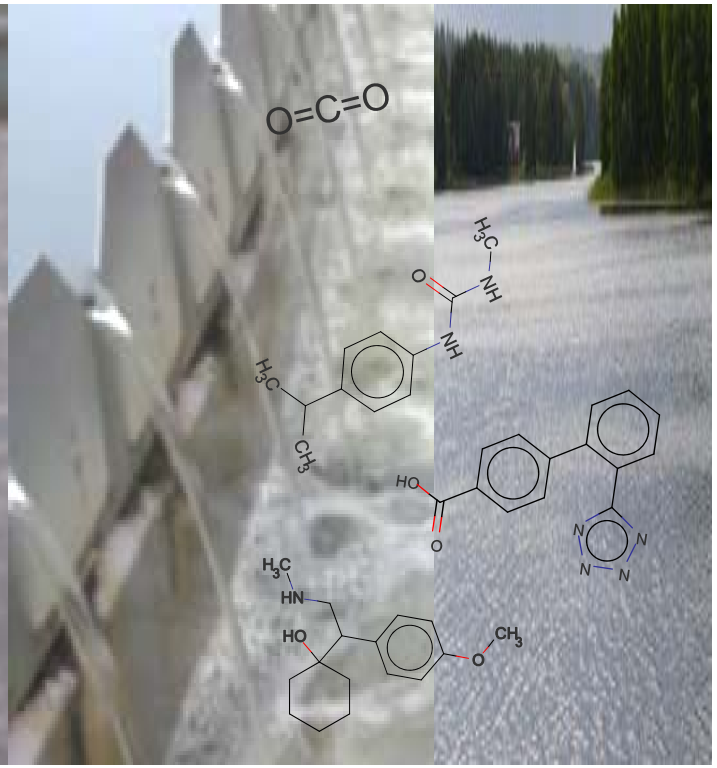
Background

Fate of micropollutants (MPs) in Wastewater Treatment Plants

MPs | Activated sludge communities | Discharge



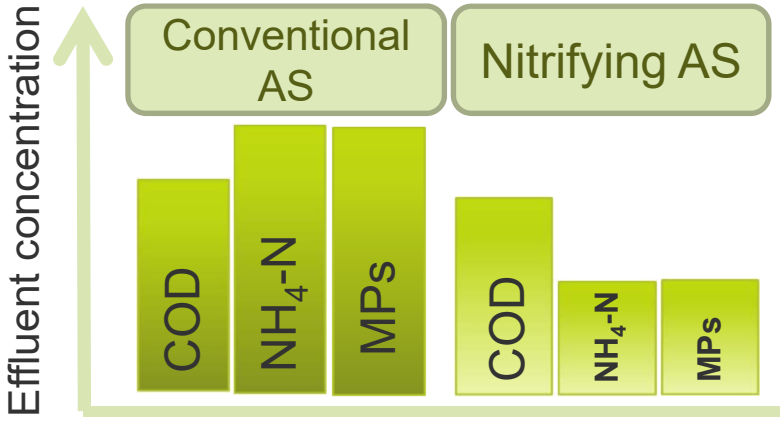


- Persistent or biodegradable?
- Compete or incomplete transformation?
- Ecotoxicity of parant compounds and transformation products?

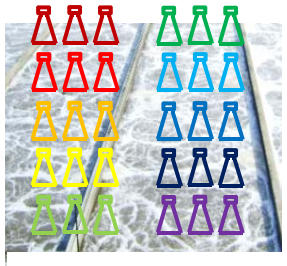
Background

Micropollutant biotransformation by activated sludge communities

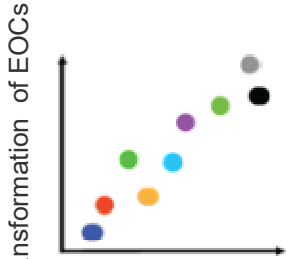
Iopromide Trimethoprim	Batt *et al*., 2006
Diclofenac Naproxen Carbamazepine Propyphenazone	Tran *et al*., 2009
EE2	Khunjar et *al*., 2011
Atenolol	Sathyamoorthy *et al*., 2013
A variety of PPCPs	Kreuzinger *et al*., 2005; Fernandez-Fontaina *et al*., 2012; Rattier *et al*., 2004

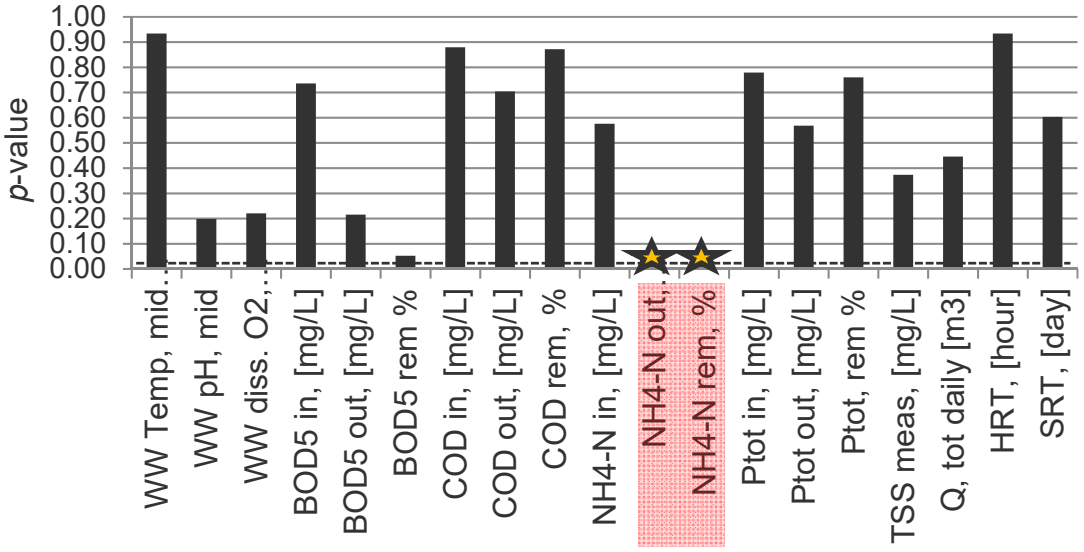


10 different WWTPs



10 diverse MPs

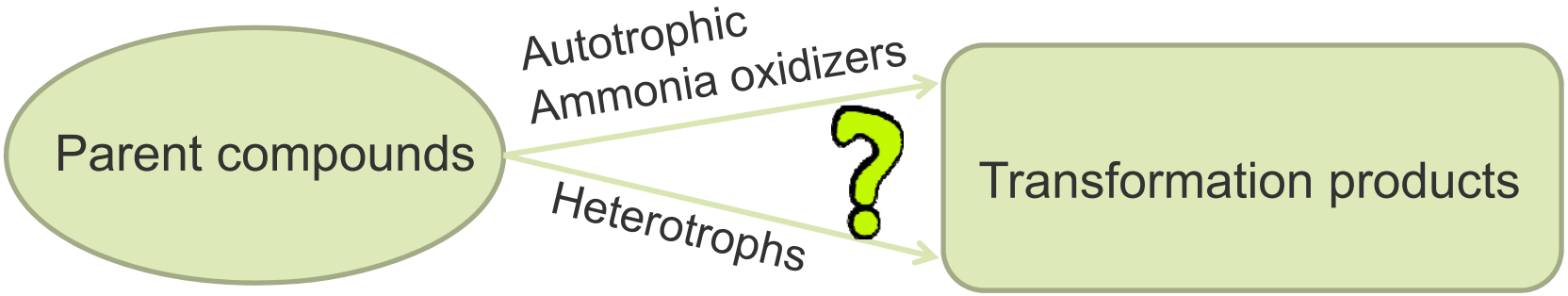




(Helbling *et al*., 2012. ES&T)

Hypothesis

Causal relationship between ammonia oxidizers and MP biotransformation



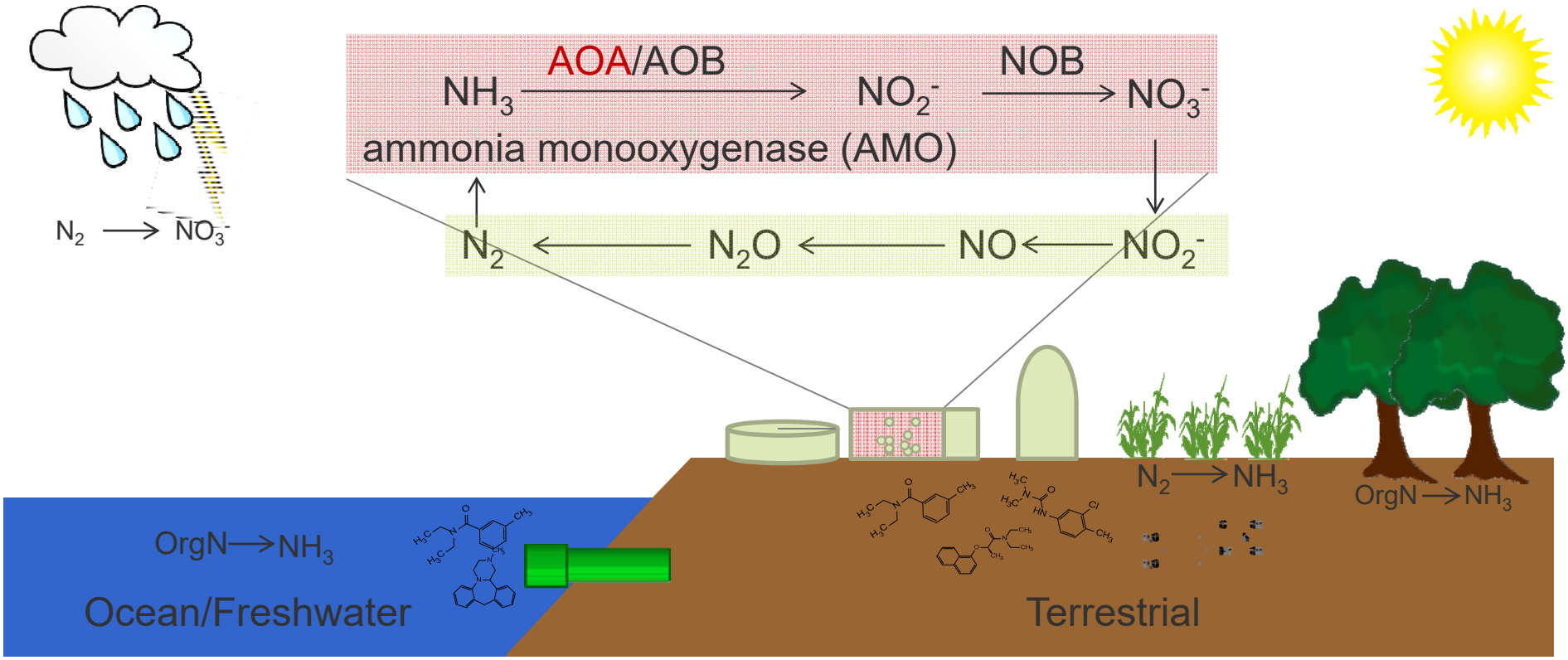
To test the hypothesis

- ❑ Pure culture study
  - ✓ **A**mmonia-**O**xidizing **B**acteria (AOB)
  - ? **Ammonia-Oxidizing Archaea (AOA)**
- ❑ Inhibition study
- ❑ Removal in full-scale WWTPs

MP biotransformation by an AOA pure culture

Why AOA?

- Distribution and abundance
- Distinct physiological characteristics
- Unknown ecological significance in MP biotransformation



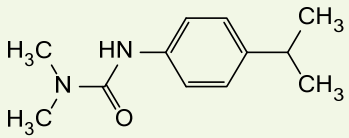
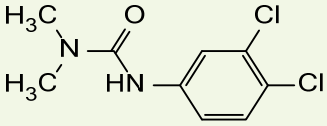
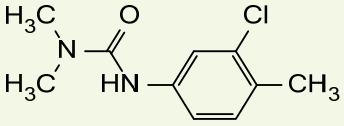
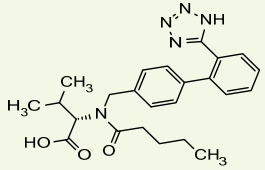
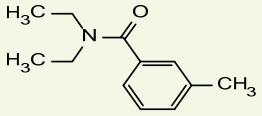
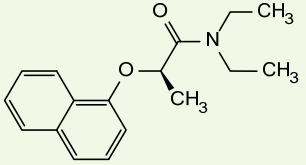
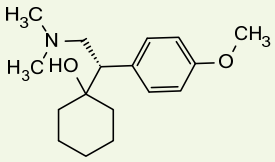
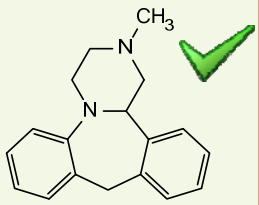
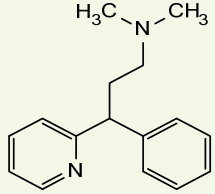
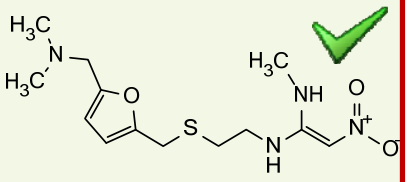
MP biotransformation capability of AOA

- **Tested ammonia oxidizers:**

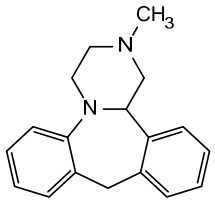
  AOA: ***Nitrososphaera gargensis***

  AOB: ***Nitrosomonas nitrosa* strain Nm90, Nm95**

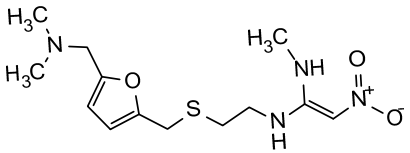
- **Tested MPs (40 µg/L each):**

Phenyl ureas		
 Isoproturon (ISO)	 Diuron (DIU)	 Chlortoluron (CHL)
Tertiary amides		
 Valsartan (VAL)	 Diethyltoluamide (DEET)	 Napropamide (NAP)
Tertiary amines		
 Venlafaxine (VEN)	 Mianserin (MIA)	 Pheniramine (PHE)	 Ranitidine (RAN)

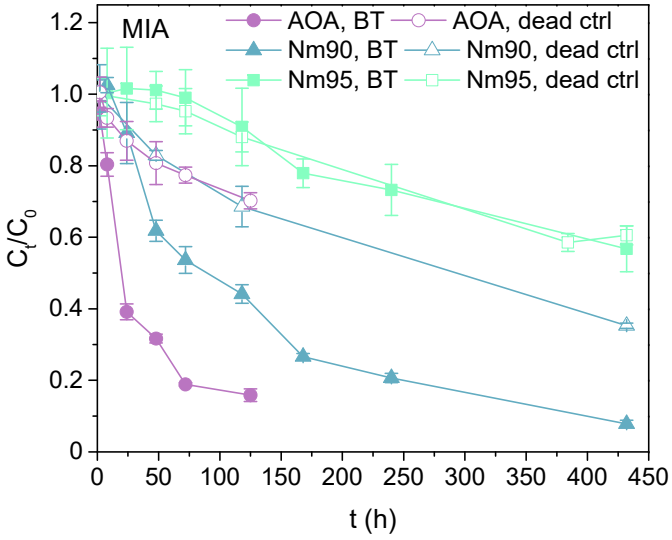
Two MPs (MIA and RAN) were biotransformed by AOA

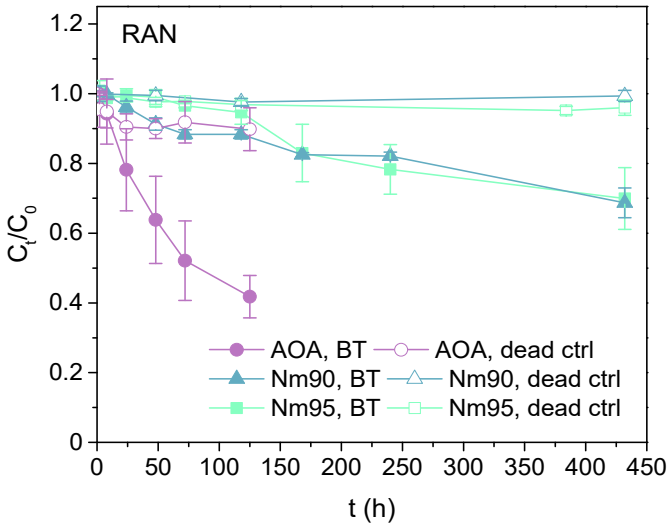


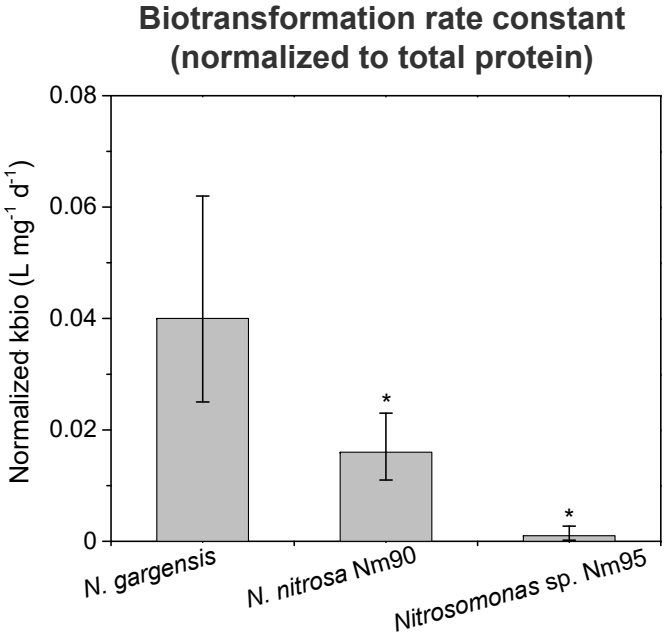
MIA

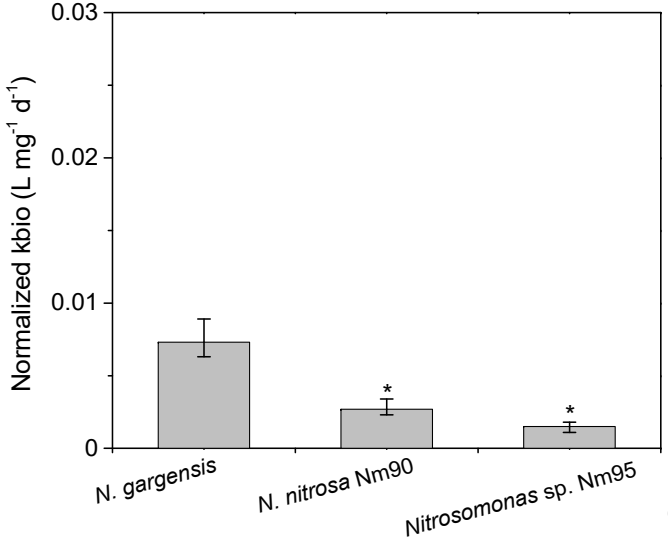


RAN



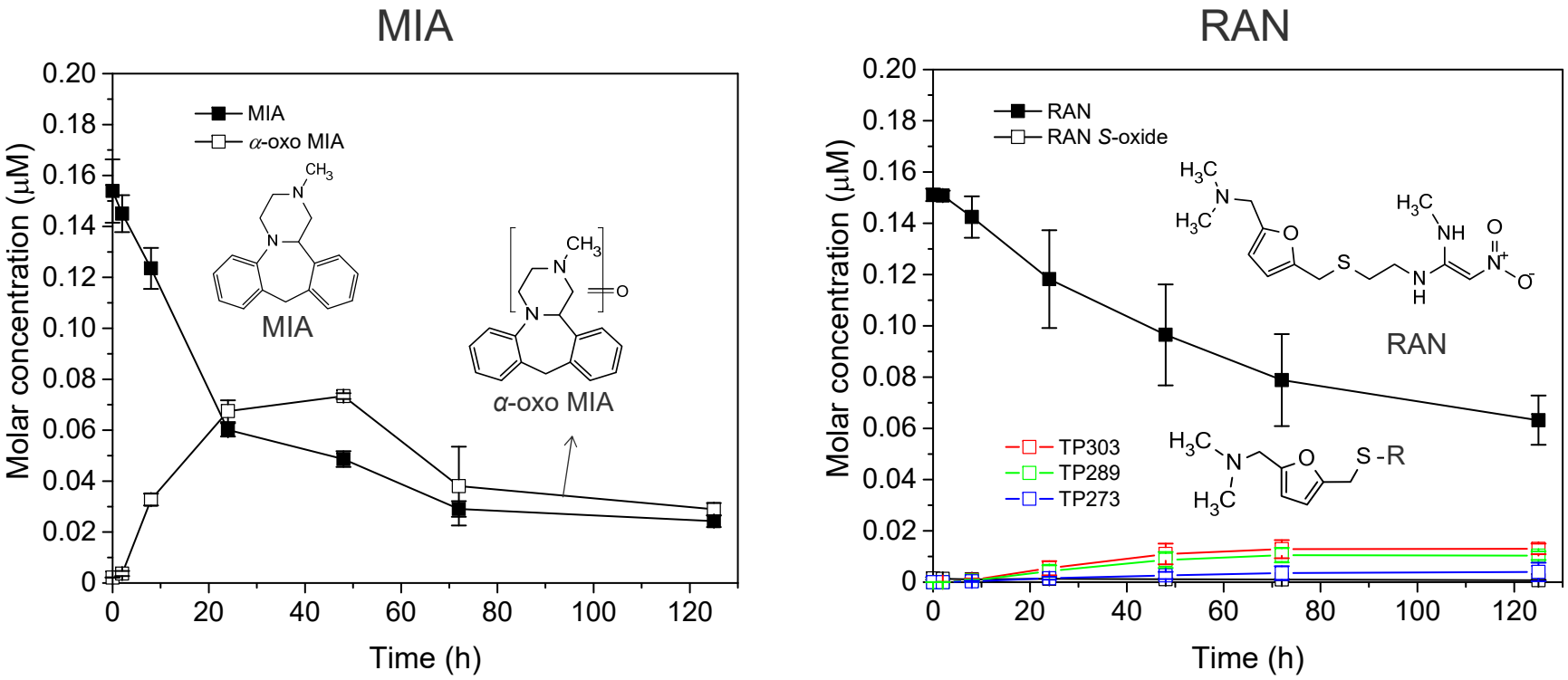






Transformation product (TP) identification

- One TP (*m/z* 279.1492) of MIA was determined to be ***α*-oxo mianserin**.
- No *N*-oxidation (mianserin *N*-oxide) or *N*-demethylation (normianserin)
- No suspected RAN TP was detected, except RAN *S*-oxide
- Three RAN TPs from non target analysis



(TPs were semi-quantified according to peak areas)

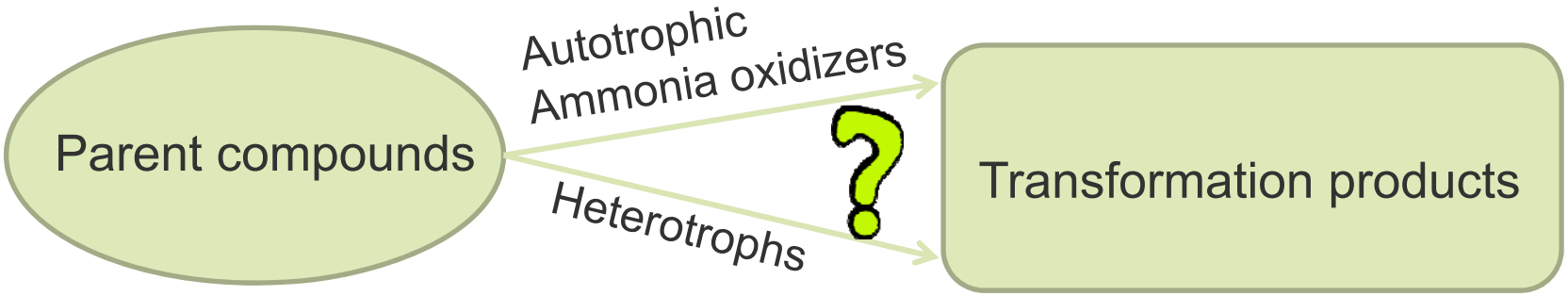
(Men *et al.*, 2016. ES&T)

Summary — Pure culture study

- ✓ *N. gargensis* (an AOA strain) can biotransform two out of the ten tested MPs: mianserin (MIA) and ranitidine (RAN).
- ✓ α-oxo mianserin was the major MIA TP.
- ✓ Three TPs were identified for RAN from non-target analysis.
- ✓ Biotransformation of MIA and RAN by AOA was cometabolic .

Hypothesis

Causal relationship between ammonia oxidizers and MP biotransformation



To test the hypothesis

- ❑ Pure culture study
  - ✓ AOB
  - ✓ AOA
- ❑ Inhibition study
- ❑ Removal in full-scale WWTPs

Inhibition study

Nitrifying **A**ctivated **S**ludge (NAS)

Ctrl

Inhibitor

AUR: **A**mmonia **U**ptake **R**ate

Hypothesis:

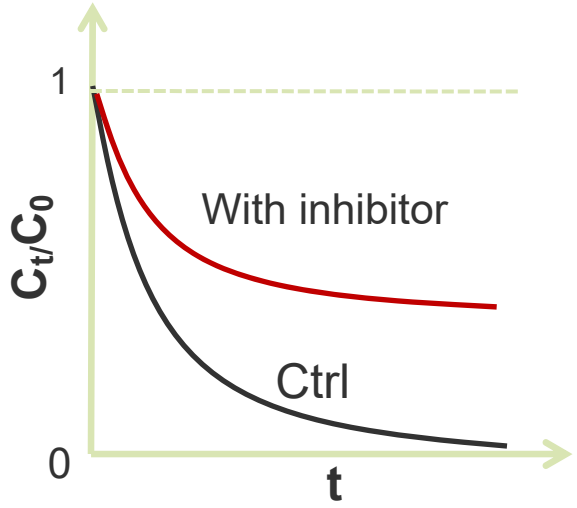
Ammonia oxidizers (AOB and/or AOA)

NH_4-N ↑ ⟶ NO_3-N ↓

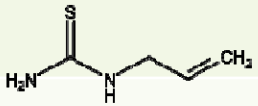
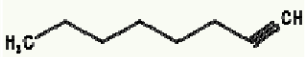
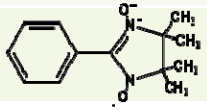
MPs ↑ ⟶ TPs ↓

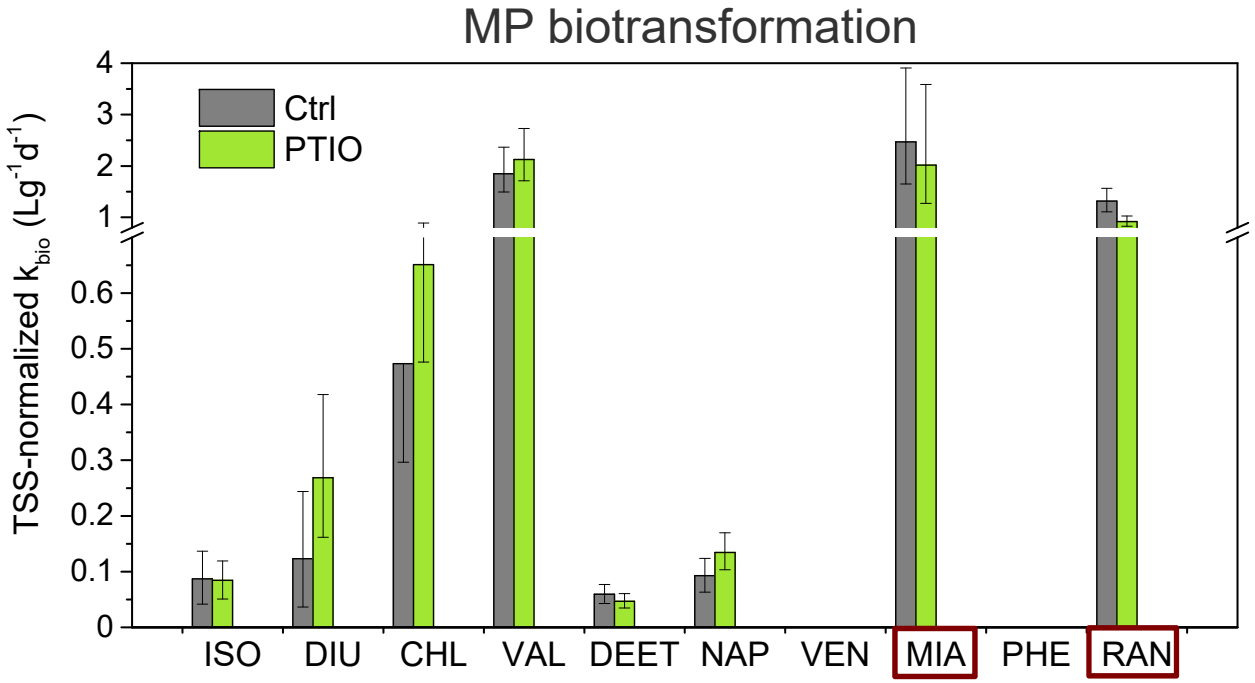
Heterotrophs

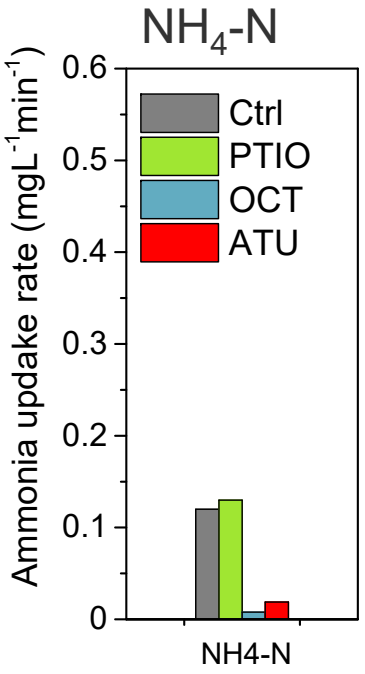
MPs ⟶ TPs



Roles of AOA in MP biotransformation

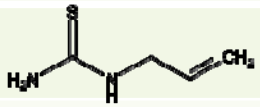
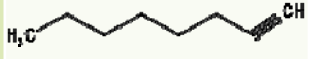
Inhibitor	*Structure*	*Conc.*	*Inhibition effeciency*	*Inhibition mechanism*	*Ref.*
Allylthiourea (ATU)		**170 μM**	AOB: 100% AOA: partial/none	Cu chelator	Ginestet *et al*. 1998, Jantti *et al*. 2013
Octyne (OCT)		**20 μM C_{aq}**	AOB: 100% AOA: partial/none	Covalent binding to AMO	Taylor *et al*., 2013
PTIO		**100 μM**	AOB: none AOA: 100%	NO scavenger	Martens-Habbena *et al*., 2015

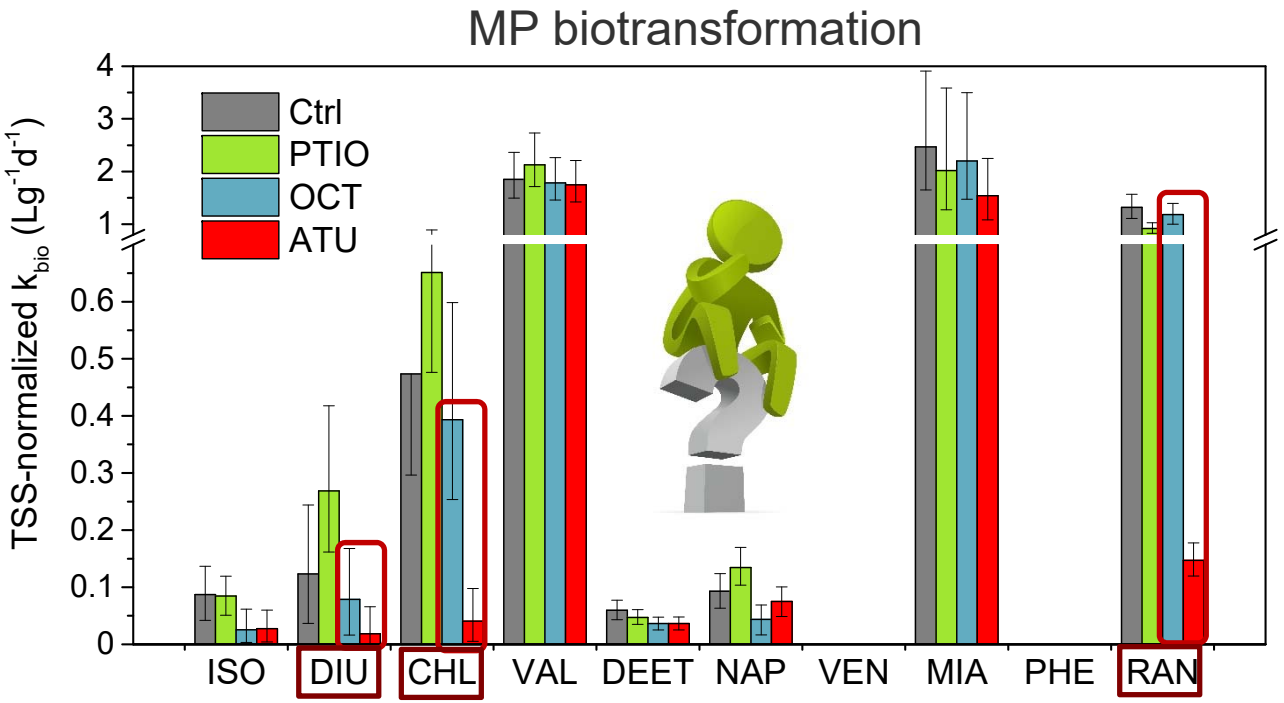


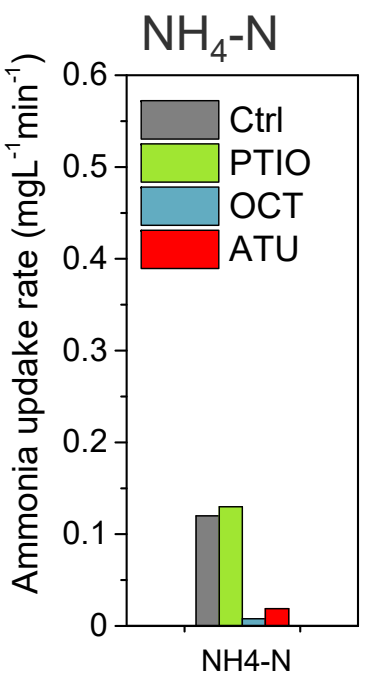


(Men *et al*., 2016. ES&T)

ATU and OCT exhibited different inhibition on AOB

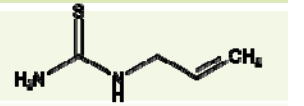
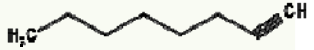
Inhibitor	*Structure*	*Conc.*	*Inhibition effeciency*	*Inhibition mechanism*	*Ref.*
Allylthiourea (ATU)		**170 μM**	AOB: 100% AOA: partial/none	Cu chelator	Ginestet *et al*. 1998, Jantti *et al*. 2013
Octyne (OCT)		**20 μM C_{aq}**	AOB: 100% AOA: partial/none	Covalent binding to AMO	Taylor *et al*., 2013

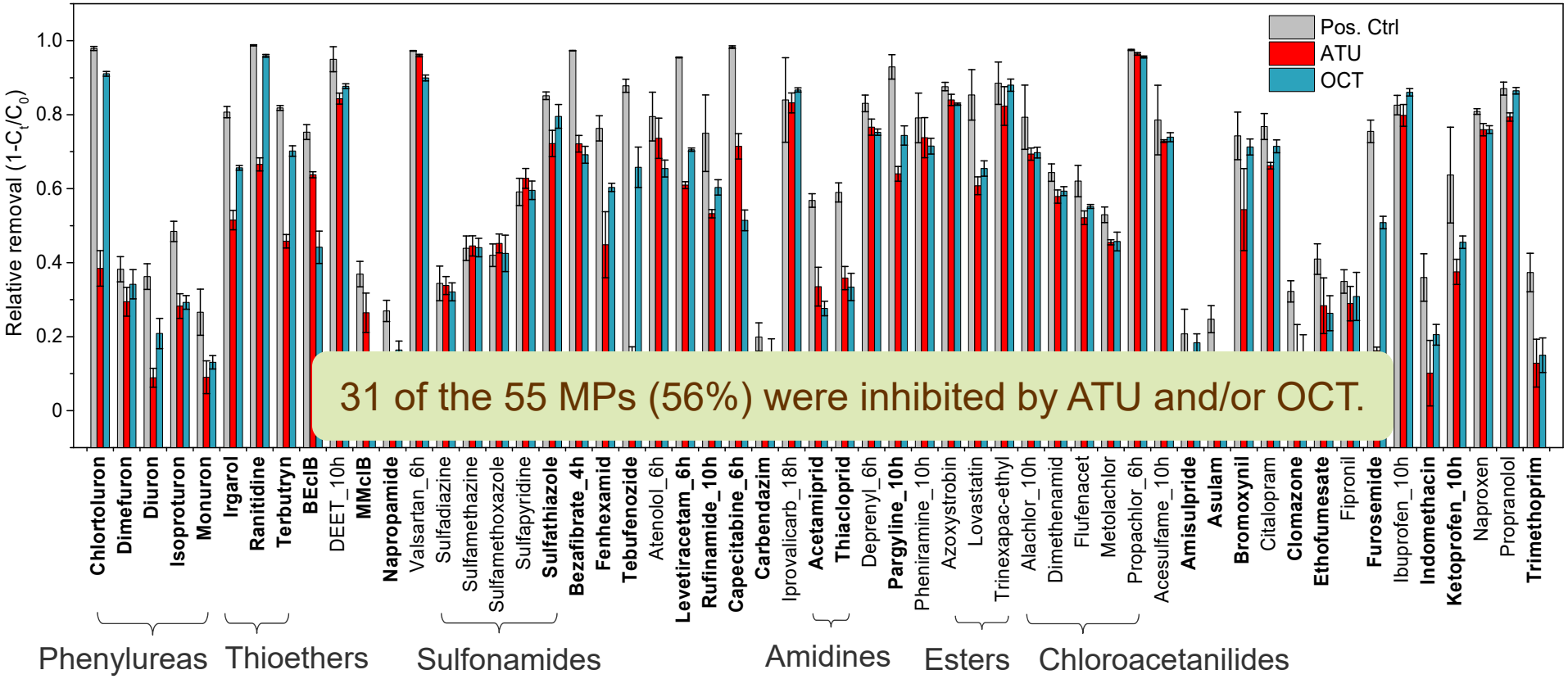




ATU exhibited higher inhibition for an extended MP list

Relative Removal > 20%	Relative removal <20%	Strong sorption & fast abiotic removal	*Total*
55	20	4	*79*

AOB Inhibitors	*Structure*
Allylthiourea (ATU)	
Octyne (OCT)	

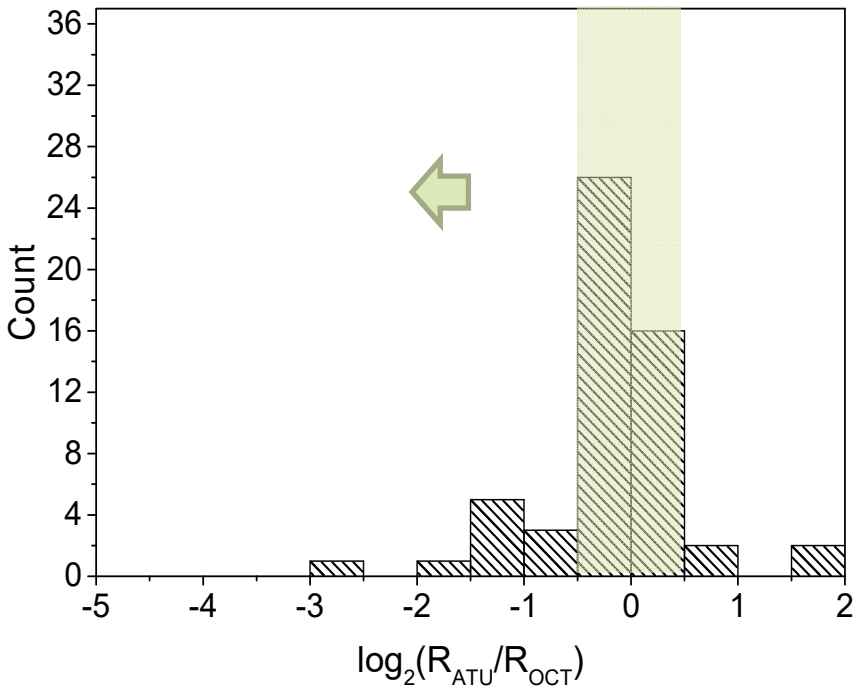


31 of the 55 MPs (56%) were inhibited by ATU and/or OCT.

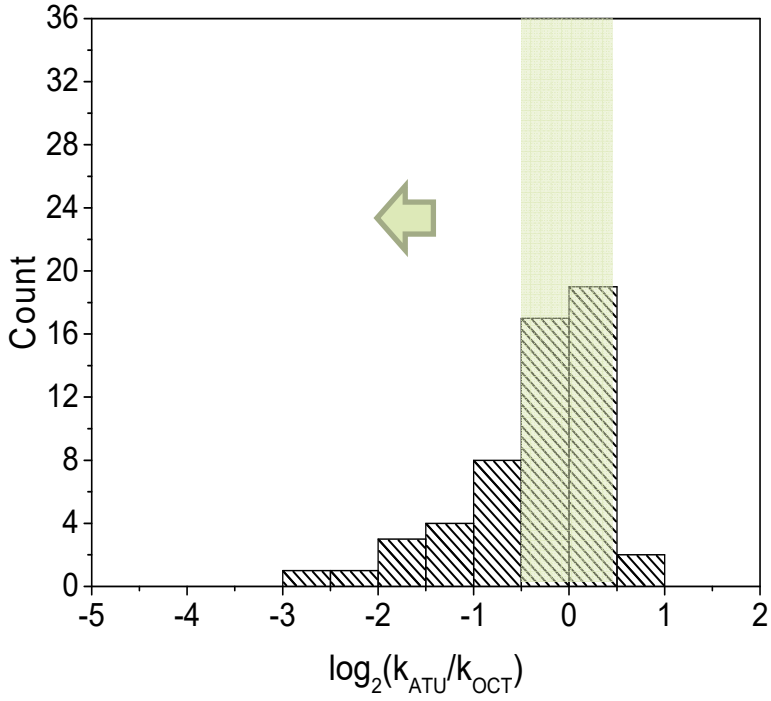
17 MPs with higher inhibition by ATU than OCT

ATU vs OCT

Removal (R)



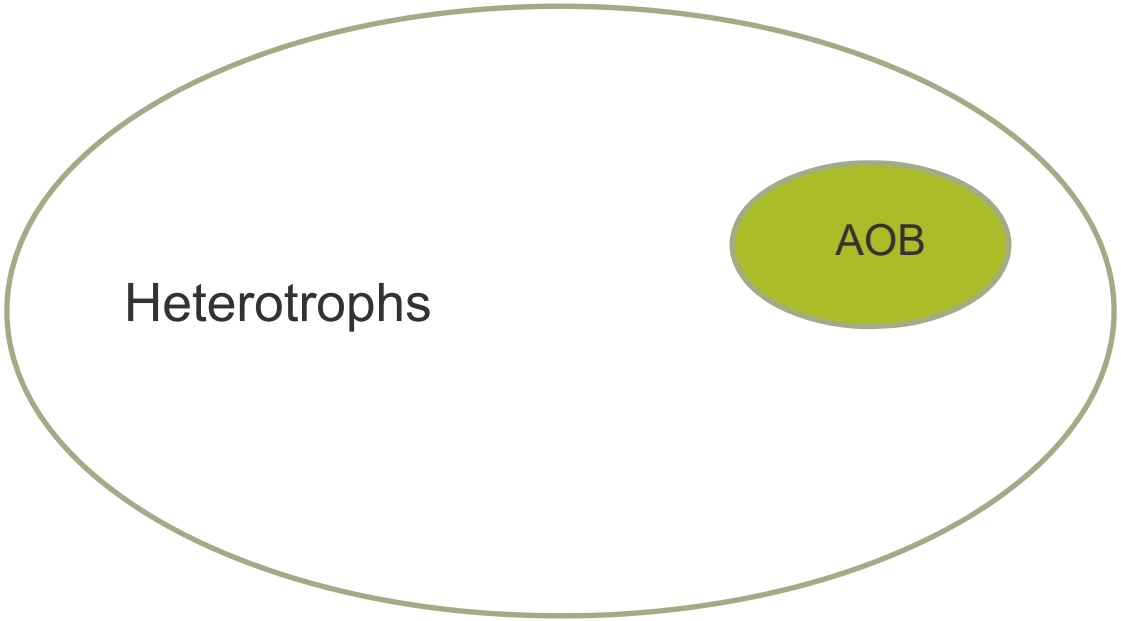
Rate constant (k), h^{-1}



Roles played by different NAS community members

Previous hypothesis: ATU is a specific AOB inhibitor.

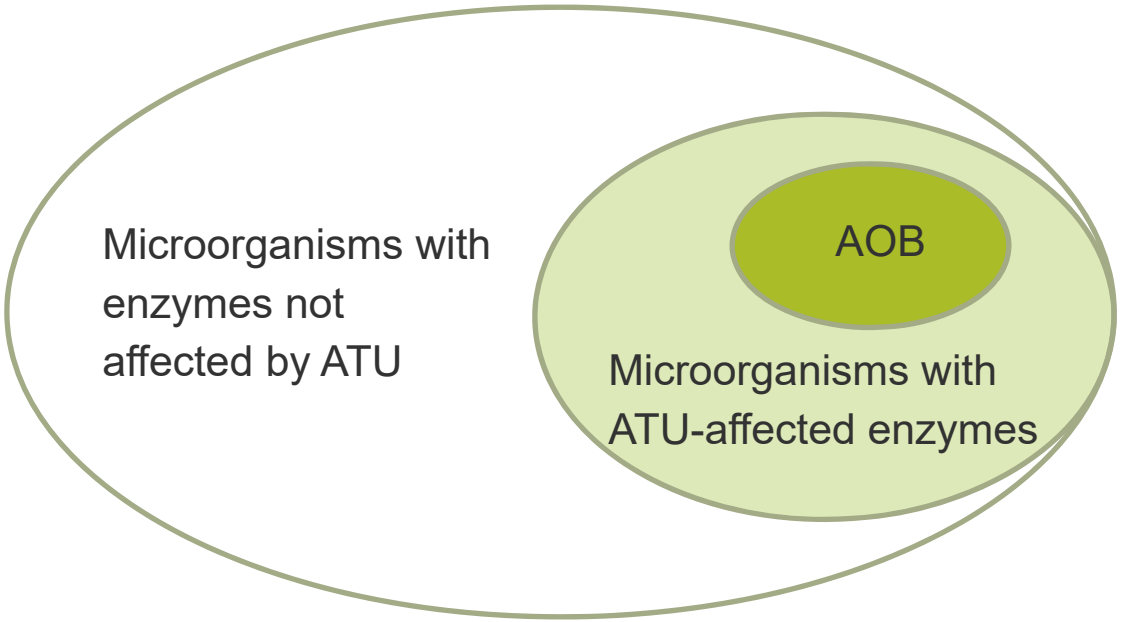
Classification of members in a NAS community based on ATU sensitivity



Roles played by different NAS community members

This study: ATU does not specifically inhibit AOB, and other ATU-affected enzymes might also contribute to the biotransformation of some MPs.

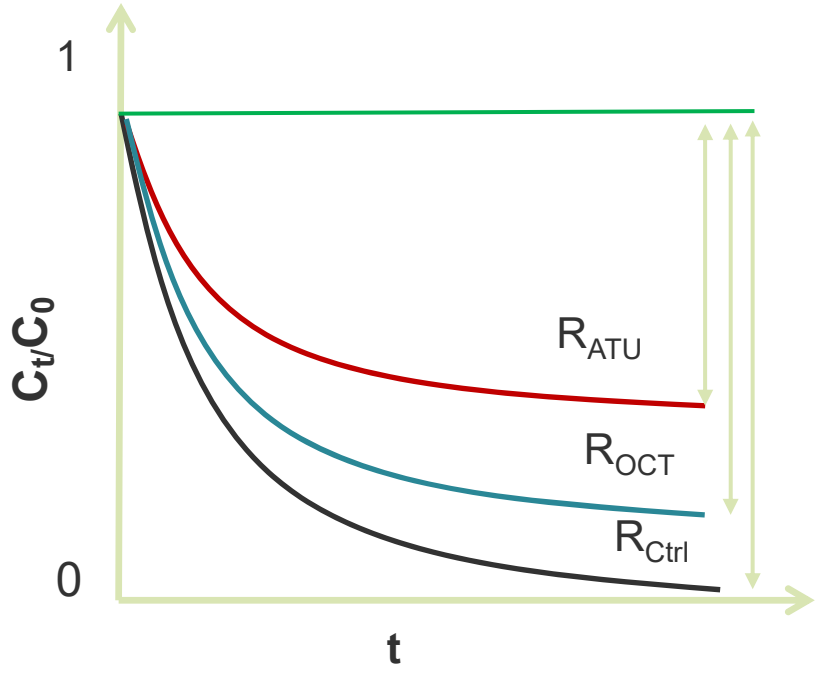
Refined Classification of members in a NAS community based on ATU sensitivity



Comparing inhibition effects of ATU and OCT

AOB	Other ATU-affected microbes	ATU-unaffected microbes
Similar inhibition	Higher inhibition by ATU	No inhibition

Contribution of the three microbial groups



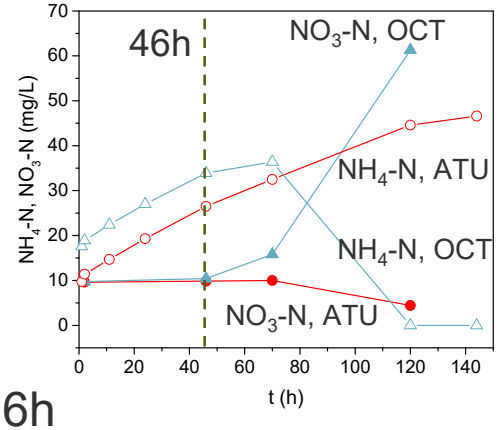
Extent of inhibition (*I*) = $\frac{R_{Ctrl}-R_{Treated}}{R_{Ctrl}}$,

R: relative removal (1-C_t/C_0)

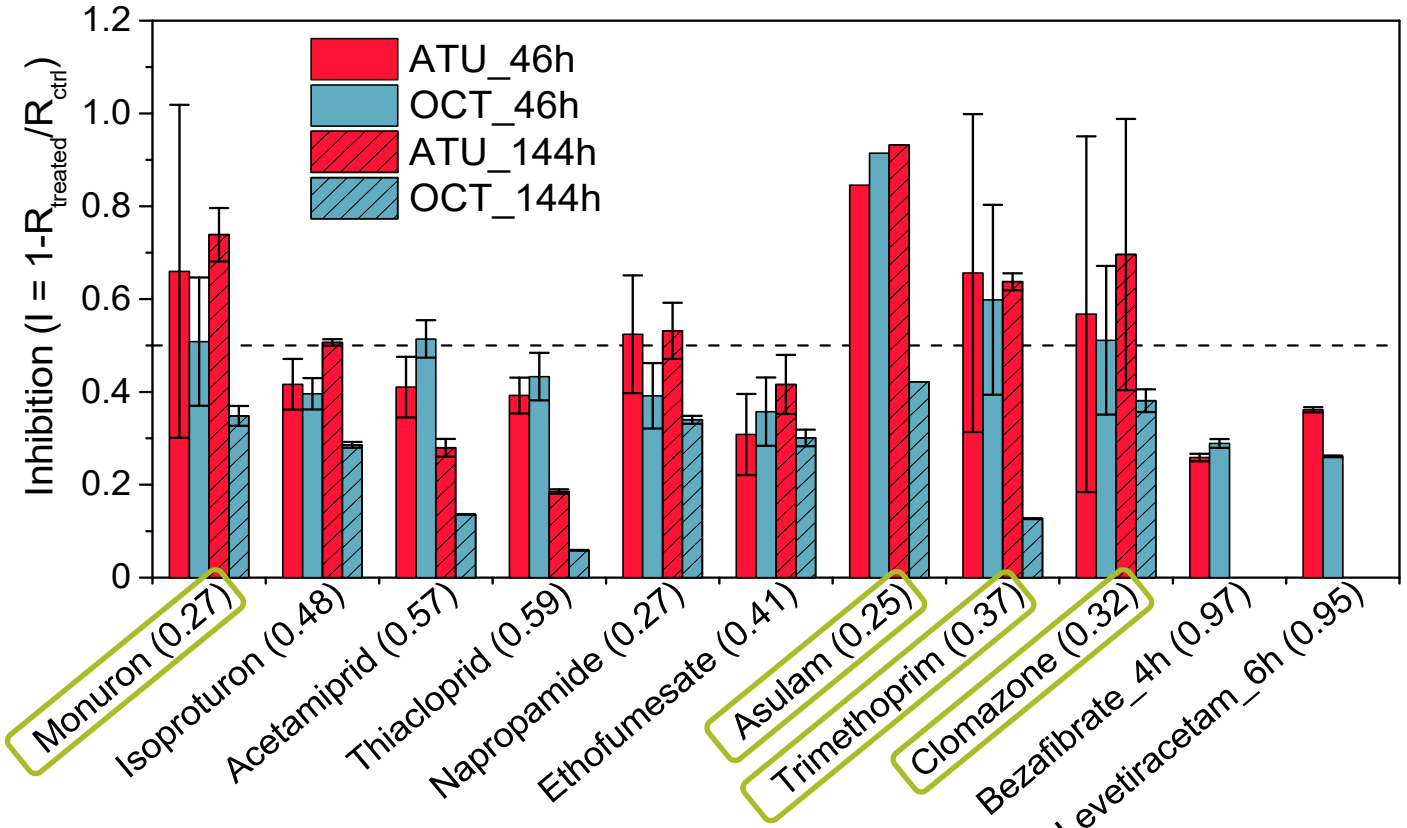
I : how much the inhibited member contribute to MP biotransformation

11 MPs with similar inhibition by ATU and OCT

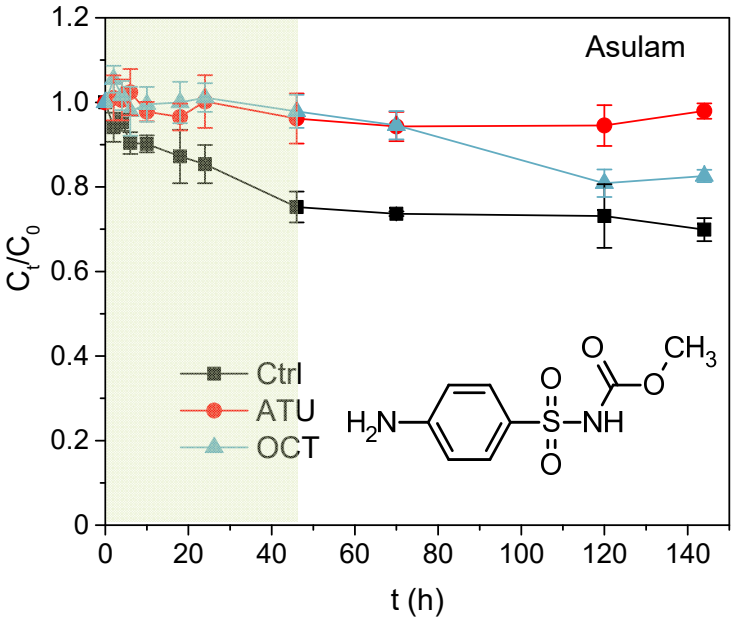
Recovery of nitrification after 46h in OCT-treated samples

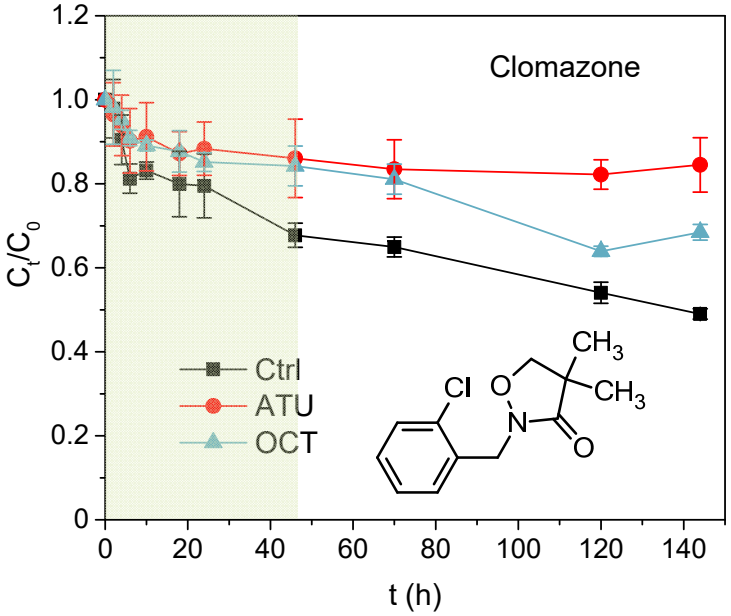


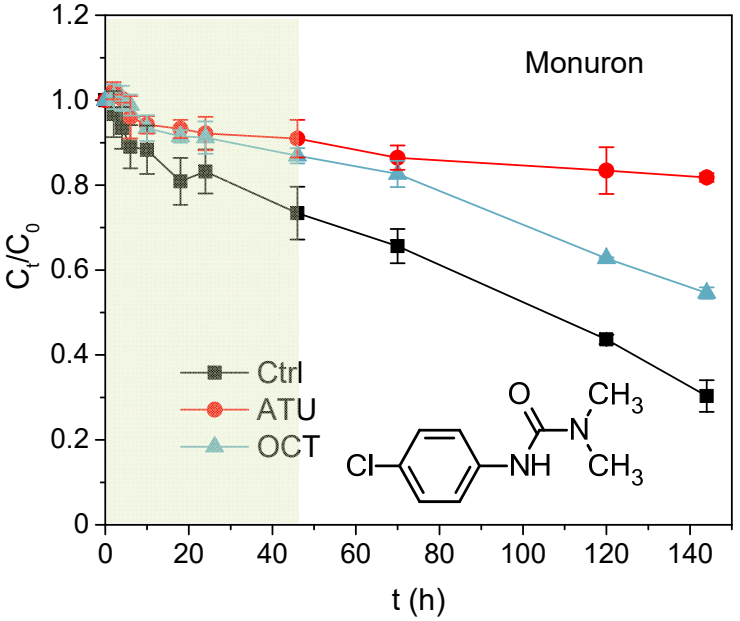
Recovery of biotransformation for the 11 Compounds after 46h

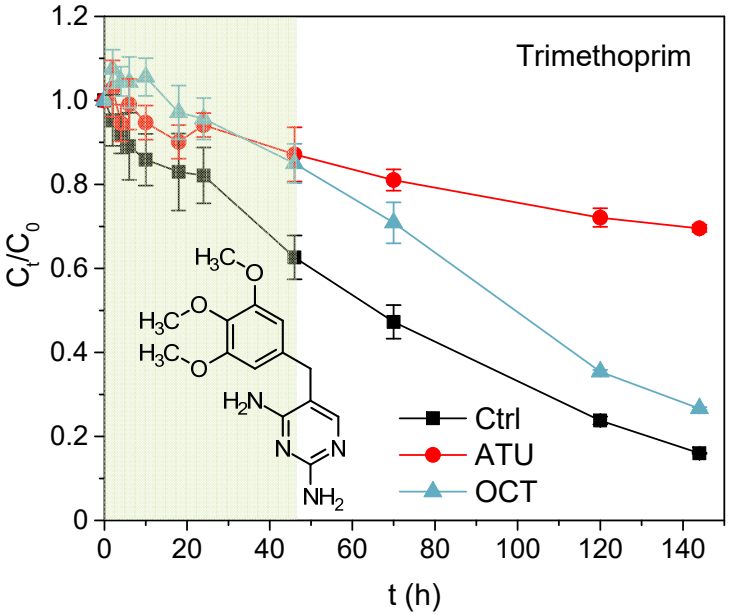


AOB played a major role in the biotransformation of 4 MPs

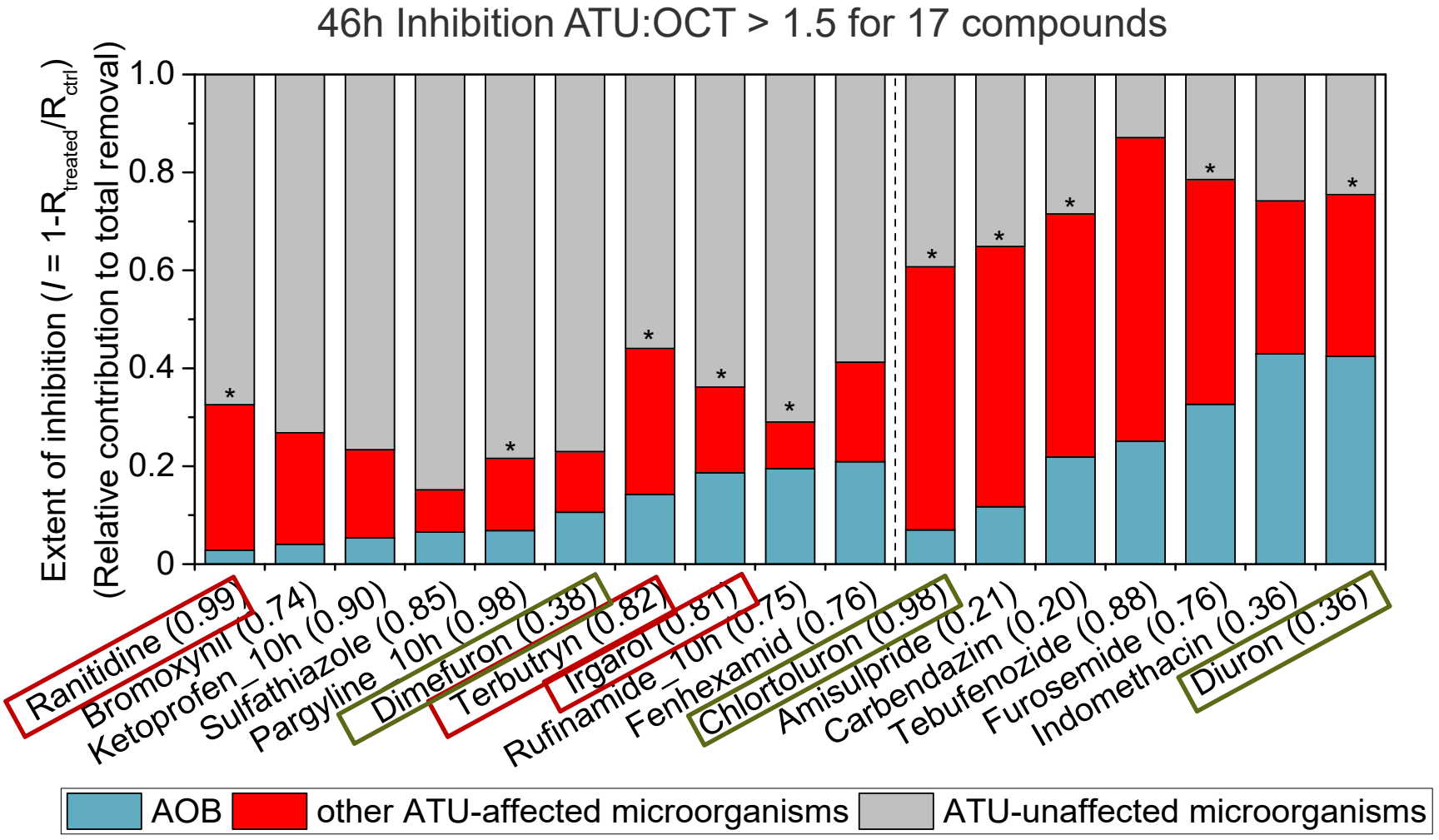








17 MPs with higher inhibition by ATU than OCT

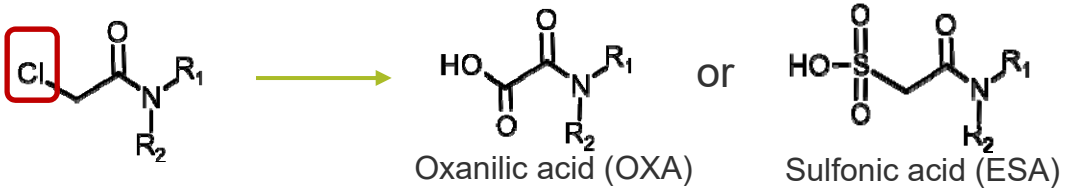


Number in parentheses (relative removal of the control at 46h unless specified)
* indicates statistically significant difference with t-test and BH correction (FDR < 0.05)

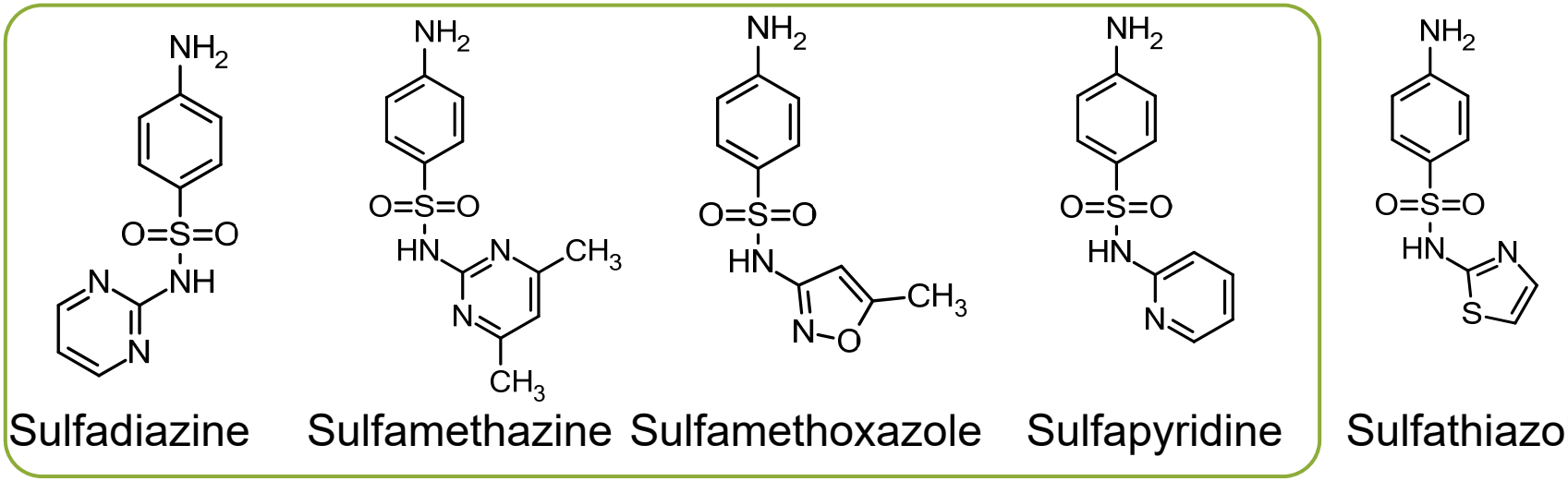
phenylurea pesticides thioethers

(Men *et al.*, Water Res.)

No inhibition by the two AOB inhibitors for the following MPs:

- Esters: hydrolysis
- Chloroacetanilides: 

- Sulfonamides: hydroxylation on aromatic ring



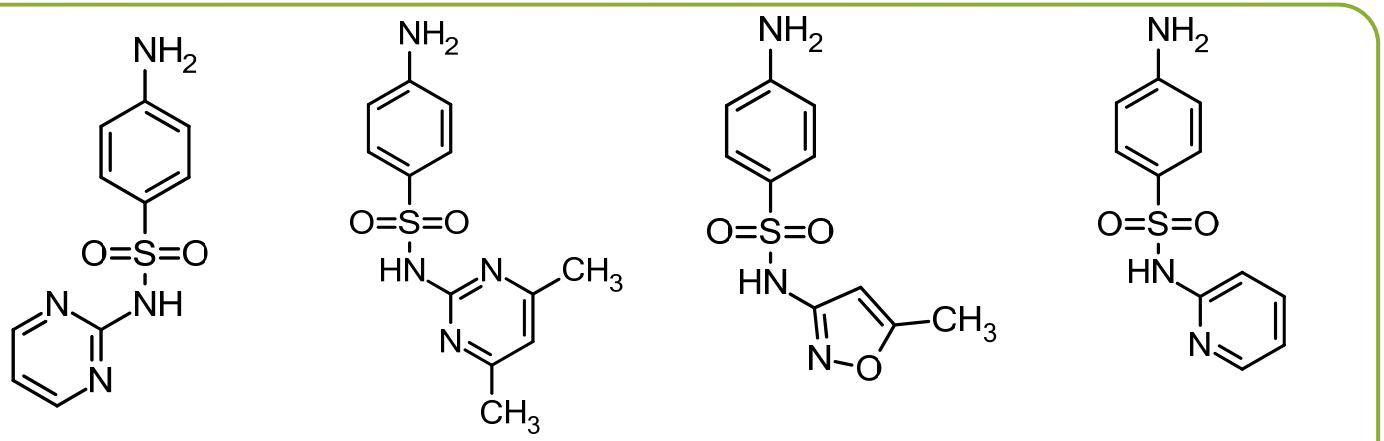
No inhibition

Inhibited by ATU
(~ 15%)

Compound specificity in AOB-related biotransformation

Phenylurea pesticides
Thioethers

Sulfonamide antibiotics



No inhibition

Inhibited by ATU
(~ 15 %)

Comparison with ATU inhibition studies in literature

MP	ATU added (mg/L)	TSS (g/L)	NH_4-N added (mg/L)	MP conc. (µg/L)	I_{ATU} in reference[a]	I_{ATU} (I_{OCT}) in this study[b]	Reference
Ibuprofen	10	1	100	100	0.25	0.03 (0)	(Tran et al., 2009)
Ibuprofen	10	1[e]	70/140	2000	0	0.03 (0)	(Roh et al., 2009)
Ibuprofen	12	1.8-5.1	<2/10/29	100	0-0.47	0.03 (0)	(Falas et al., 2012)
Atenolol[c]	30	1.73	20	15	0.63	0.04 (0.06)	(Sathyamoorthy et al., 2013)
Bezafibrate	50	4-16	20	1	0.48	0.26 (0.29)	(Maeng et al., 2013)
Ketoprofen	5	NA	0-0.5	2	0.76	0.41 (0.28)	(Rattier et al., 2014)
Ketoprofen	10	1	100	100	0.57	0.41 (0.28)	(Tran et al., 2009)
Ketoprofen	50	4-16	20	1	0.75	0.41 (0.28)	(Maeng et al., 2013)
Diclofenac[d]	10	1	100	100	0.67	0.97 (0.58)	(Tran et al., 2009)
Diclofenac	50	4-16	20	1	0.32	0.97 (0.58)	(Maeng et al., 2013)
Furosemide	5	NA	0-0.5	2	0.78	0.79 (0.33)	(Rattier et al., 2014)
Indomethacin	10	1	100	100	0.86	0.74 (0.43)	(Tran et al., 2009)
Irgarol	11.1	2.5-4.7	50	1	0.37	0.36 (0.19)	(Margot et al., 2016)
Terbutryn	11.1	2.5-4.7	50	1	0.53	0.44 (0.14)	(Margot et al., 2016)
Trimethoprim	5	3.3	50	250	0.64	0.66 (0.60)	(Batt et al., 2006)
Trimethoprim	5	NA	0-0.5	2	<0	0.66 (0.60)	(Rattier et al., 2014)
Trimethoprim	10	NA	~24	0.5	0	0.66 (0.60)	(Khunjar et al., 2011)

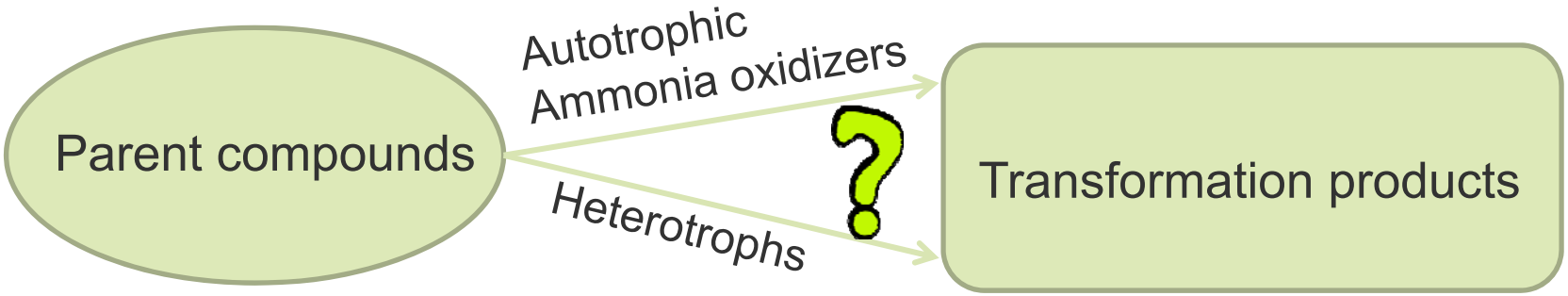
(Men *et al.*, Water Res.)

Insights into roles of AOB in MP biotransformation

Microorganisms with enzymes not affected by ATU	Microorganisms with ATU-affected enzymes other than AOB	AOB
This study		
• Esters • Chloroacetanilides • Sulfonamide antibiotics (except sulfathiazole) • Others	• Phenylureas • Thioethers • Others	• Asulam • Clomazone • Monuron • Trimethoprim
Literature		
• ✓ Ibuprofen • ✓ Naproxen • ✓ Valsartan • ✓ DEET • ✓ Trimethoprim		• ✓ Trimethoprim • Ketoprofen • Furosemide • Indomethacin • Irgarol • Terbutryn

Hypothesis

Causal relationship between ammonia oxidizers and MP biotransformation

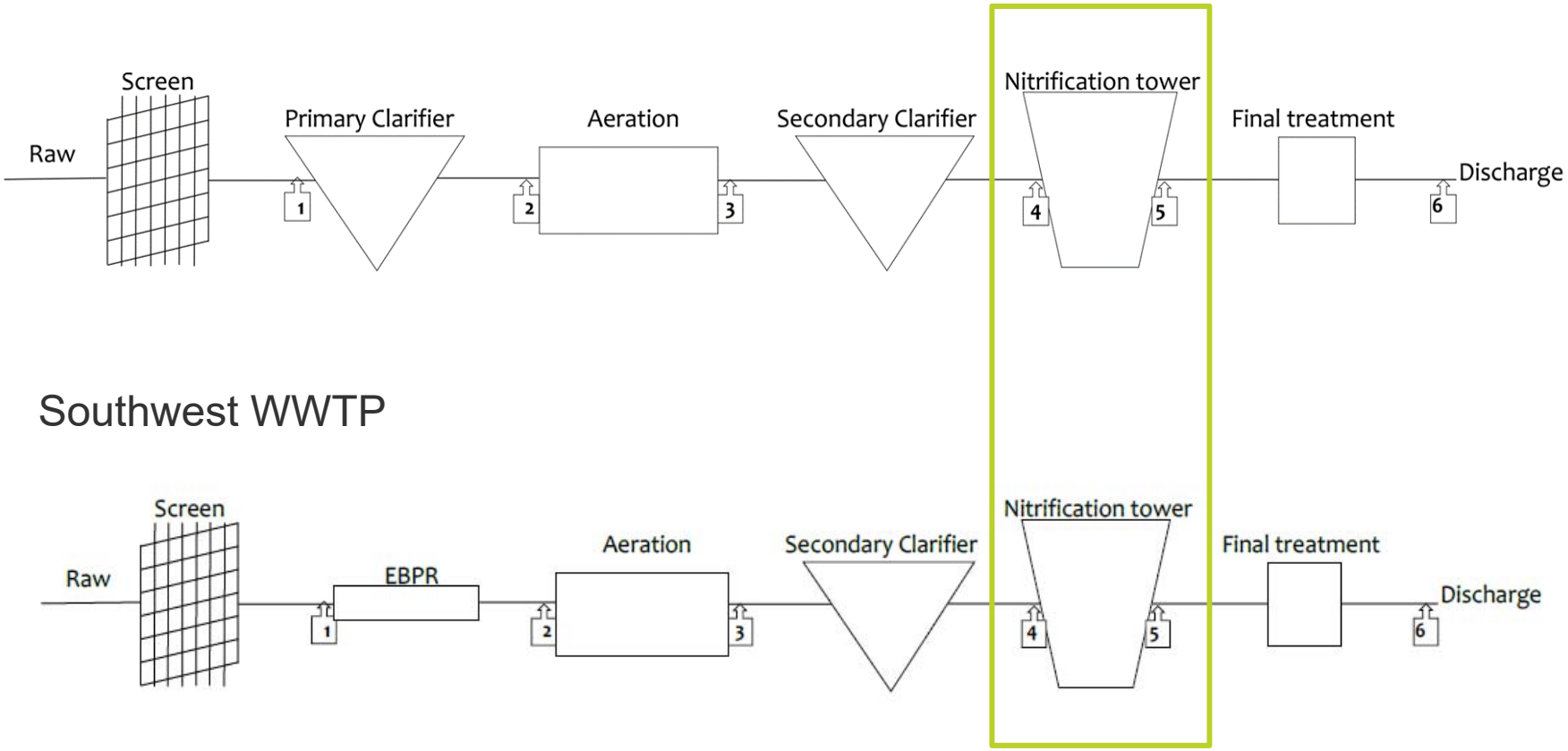


To test the hypothesis

- ❑ Pure culture study
  - ✓ AOB
  - ✓ AOA
- ❑ Inhibition study
- ❑ Removal in full-scale WWTPs

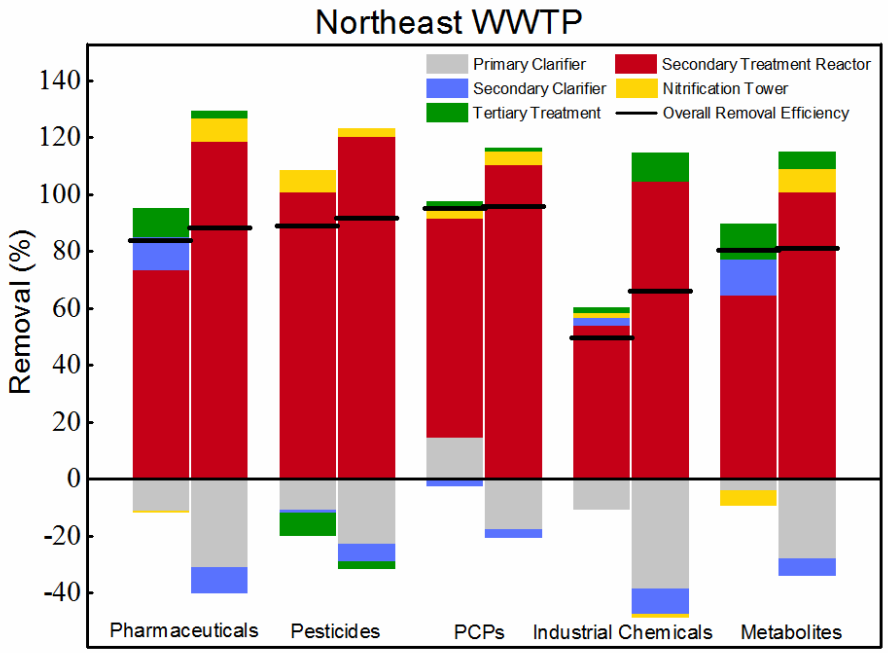
Roles of additional nitrification treatment (nitrification tower) in MP biotransformation

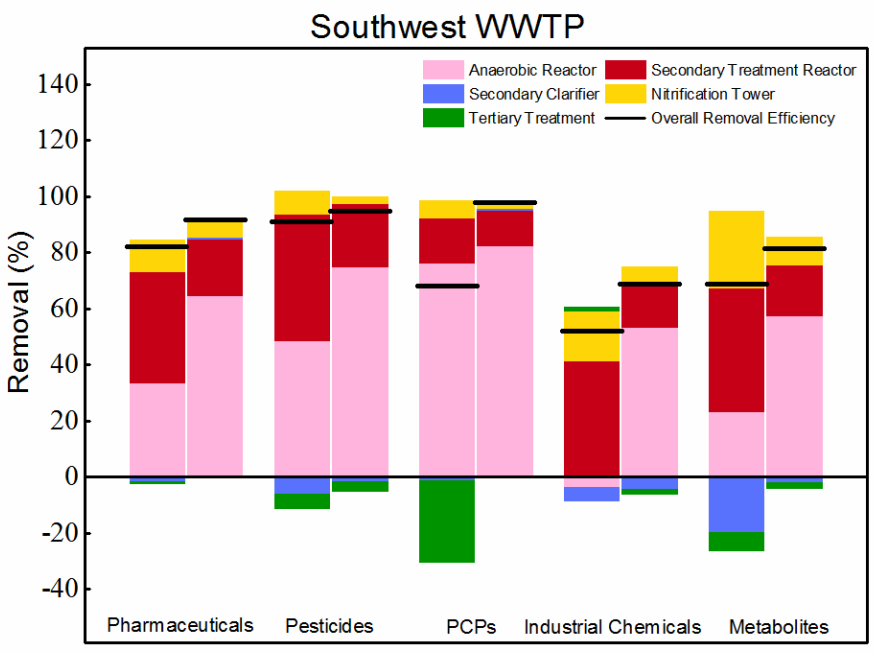
Northeast WWTP



Southwest WWTP

MP removal in each treatment step





Concluding Remarks

- ✓ AOB and AOA species were able to biotransform mianserin and ranitidine, likely via cometabolism by AMO.
- ✓ ATU is not a specific inhibitor to study the roles of ammonia oxidizers in NAS. Roles of AOB in MP biotransformation seem to be overestimated in inhibition studies using ATU. More specific inhibitors should be considered.
- ✓ AOB in NAS played a major role in the biotransformation of four compounds (i.e. asulam, clomazone, monuron, and trimethoprim).
- ✓ Other microbes with ATU-affected enzymes were strongly involved in the biotransformation of a number of MPs, including thioethers and phenylureas.
- ✓ AOB and microorganisms with ATU-affected enzymes were not involved in the biotransformation of esters, chloroacetanilides and sulfonamide antibiotics (except for sulfathiazole) investigated in this study.
- ✓ MP biotransformation was carried out by multiple community members in NAS. TP identification can further help differentiate roles of community members.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Thank you for your attention!